A Global Perspective on the History, Use, and Identification of Synthetic Food Dyes

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The use of synthetic food colorants is widespread both in the United States and around the world. While these food dyes have no nutritional value, they do brighten our soft drinks, snacks and desserts, and we all know that the appearance of food is very important *(1–3)*. Most chemistry teachers use real-world examples to help connect chemical concepts to students' every-day lives as evidenced by numerous articles in virtually all recent issues of this *Journal*. In fact, connecting chemical concepts with relevant examples has become commonplace *(4)*. According to constructivist learning theory, knowledge is constructed in the mind of the learner, and if learners have relevant, tangible examples of chemical concepts, they are much more likely to find meaning in these concepts *(5)*. The use of real-world examples helps motivate students and maintain their interest in learning chemistry *(6)*. The Chemistry in the Community (ChemCom) curriculum and, more recently, the ChemConnections modular materials have both been developed around this concept and evaluations demonstrate that it helps students learn *(7, 8)*.

The discussion of food dyes is an excellent way to introduce students to the extremely common and very useful field of chromatography. The separation and identification of these dyes using paper chromatography has been widely used by science teachers and chemistry instructors for many years because the experiments are simple, safe, and inexpensive. Because many students consume foods containing these dyes on a daily basis, they are personally connected and interested in the topic *(9–11)*. This *Journal* has also published works involving other forms of chromatography for dye separation and identification, including thin layer *(12)*, high performance liquid *(13)*, and column chromatography *(14, 15)*. The identification of synthetic food dyes using visible spectroscopy is also a commonly performed experiment by chemistry students *(12, 16–20)*.

This paper presents a brief history of the development and use of synthetic food colorants and provides structures, common names, and technical information for the seven synthetic U.S. Food and Drug Administration (FDA)-certified dyes, and also for seven additional synthetic food dyes commonly used outside of the United States. Inclusion of this topic would be interesting to high school students, as well as to nonscience majors in college and to those taking general or organic chemistry. Instructors will find this background information useful for introducing laboratory experiments involving the identification of dyes by paper chromatography and visible spectroscopy. On the basis of the information here, students could be able to interpret data and arrive at conclusions involving unknown mixtures of U.S. and foreign dyes. This inquiry-based approach provides "real-world" applications of paper chromatography and visible spectroscopy.

A Brief History of Artificial Food Colorants

The artificial coloration of foods is not a new phenomenon. Indeed, the use of colorants was common even in ancient times. Pliny the Elder, in the first century C.E., commented that the Gallic wine industry used smoke and aloe extract to improve the color and flavor of wines *(21)*. History documents that a variety of colorful but toxic inorganic salts such as copper sulfate, lead chromate, and mercury sulfide were used to "improve" the appearance of a wide range of foods such as butter, jams, pickles, and candy *(22)*. In the 17th and 18th centuries, as trade between Asia and Europe expanded, there was a rush to adulterate "new" foods (such as tea, coffee, and chocolate) with an ever-expanding array of colorful but toxic inorganic salts. Tea was often colored with copper carbonate, bread enhanced with chalk to increase its "whiteness", and the colors of cayenne and curry powders were adulterated with lead oxide and mercury sulfide *(23)*. The 1800s saw the rapid development and use of scientific instruments such as the balance and the microscope, from which early scientists were able to identify foreign substances in foods. Books and pamphlets began to appear criticizing the contamination of foods with potentially toxic compounds. In 1820, Frederick Accum published a seminal treatise on food adulteration *(24)*. The work of Accum is described in three extensive articles in this *Journal (25–27)*. In the United States during the mid-19th century, it was virtually impossible to find any food or drink that was not adulterated; even milk was tinted yellow with lead chromate to prevent detection of watered milk, which has a blue hue.

The materials used for coloring foods changed dramatically after the mid-1800s. In 1856, the English chemist William Perkin (1838–1907) prepared the first synthetic organic dye, "aniline purple" or "mauve", from coal tar *(28)*. Soon a wide variety of colorful, organic-based dyes began to replace toxic mineral salts as food colorants. This dramatic change in food colorants introduced potential health problems, because the safety of these organic "coal tar" dyes had not been investigated. By the end of the 19th century, the need for determining the safety of food additives in general and of food coloring agents in particular became a national concern. In 1883, Harvey W. Wiley (1844–1930), chief chemist at what is now the U.S. Department of Agriculture, began his 40-year quest to improve food safety. Known as the "Father of the Pure Food and Drugs Act", Wiley was the primary author of this act, which became law in 1906 *(29)*. One aspect of this act was designed to prevent the use of interstate commerce to ship meat and meat products that contained dyes, chemicals, or other ingredients that would render the meat unwholesome or unfit for human consumption. In addition, this act recognized seven coal tar dyes as safe for use in foods (Table 1).

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Table 1. Food, Drug, and Cosmetic (FD&C) Dyes Recognized as Safe by the U.S. FDA in 1906 *(30)*

Common Name	FDA Name
Ponceau 3R[a]	FD&C Red No. 1
Amaranth[b]	FD&C Red No. 2
Erythrosine	FD&C Red No. 3
Indigotine	FD&C Blue No. 2
Light Green SF[c]	FD&C Green No. 2
Naphthol Yellow[d]	FD&C Yellow No. 1
Orange 1[d]	FD&C Orange No. 1

[a] Delisted for food use in 1961. [b] Delisted for food use in 1976. [c] Delisted in 1966 because of "insufficient economic importance". [d] Delisted for food use in 1959.

Between 1916 and 1929, 10 dyes were added to the approved list[1] as shown in Table 2. A Federal Color Additives Law, which went into effect in July 1960, allowed continued use of these colors through "provisional" approval. Thus, Red 2, Red 3, Red 4, Blue 1, Blue 2, Green 3, Yellow 5, and Yellow 6 were now the only coal tar dyes allowed in food and food products. In 1969, Soviet scientists concluded that long-term use of Red 2 caused cancer in laboratory animals. The FDA repeated these tests with inconclusive results. Nevertheless, the FDA banned Red 2 and Red 4 in 1976 *(31, 32)*. The FDA replaced the banned Red 2 and Red 4 with another coal tar dye, Red 40, also known as Allura Red AC. Hence at the moment, the United States only permits the following seven synthetic dyes in foods: Red 3, Red 40, Yellow 5, Yellow 6, Blue 1, Blue 2, and Green 3 (see Table 3).

The safety of food dyes still remains very much an issue. Interestingly, the United States considers powdered Red 3 to be safe, but has banned the lake form[2] of this dye because it causes a carcinogenic response in rats *(33)*. Both the United States and Canada allow powdered Red 3 as a food colorant; however, it is used sparingly in the United States (see Table 3), and our research has not found it in any food colors tested from Canada. The FDA banned Red 2 in 1976 while the very same year Canada said there was insufficient evidence at the time to justify the removal of this dye from foods. Red 2 (amaranth) is still widely used around the world. The safety of Sudan I (banned in the United States in 1918)[1] came under scrutiny in Europe during the 1990s and is now banned by the European Union and China. Several studies were conducted in the 1970s that concluded that FD&C Yellow 5 and Yellow 6 cause allergic reactions and can lead to hyperactivity in children *(34, 35)*.

The European Food Safety Authority (EFSA) is currently evaluating new research that shows that certain food colors cause increased hyperactivity in children *(37)*. Food and beverage manufacturers in the United Kingdom could possibly remove Sunset Yellow (Yellow 6), Tartrazine (Yellow 5), Allura Red (Red 40), Carmoisine, Ponceau 4R, and Quinoline Yellow from their products within the next two years. This might lead the FDA to re-evaluate the safety of the seven certified FD&C dyes that are presently allowed in foods in the United States.

Table 4 lists 14 synthetic food dyes that we have found to be present in food colors from the United States, Canada, England, Ireland, France, Spain, Germany, China, Japan, Singapore, New Zealand, and Australia. While Red 4 was not identified in any of the analyzed food colors, it was included because it is still permitted in Canada. Note that dyes 10–14 do not have a FD&C name, and are not permitted in U.S. foods. Note also that dyes 3 and 9 do not have an E-number, and are banned from foods in the European Economic Community (EEC). The common names, E-numbers, CAS numbers, and Color Index (CI) numbers are given for clarity and positive identification of these most commonly used food dyes. The structures for these 14 food dyes are given in Figure 1 of the online supporting information. Table 5 lists the synthetic food dyes permitted by countries studied in this research.[3]

Table 2. FD&C Dyes Added to the Approved List, 1916–1929

Common Name	FDA Name	Year Added
Tartrazine	FD&C Yellow No. 5	1916
Sudan I	—[a]	1918
Butter Yellow	—[a]	1918
Yellow AB	FD&C Yellow No. 3[b]	1918
Yellow OB	FD&C Yellow No. 4[b]	1918
Guinea Green B	FD&C Green No. 1[c]	1922
Fast Green FCF	FD&C Green No. 3	1927
Brilliant Blue FCF	FD&C Blue No. 1	1929
Ponceau SX	FD&C Red No. 4[d]	1929
Sunset Yellow FCF	FD&C Yellow No. 6	1929

[a] Sudan I and Butter Yellow were withdrawn after being on the list for only six months after it was discovered that each caused dermatitis. Butter Yellow was later found to be carcinogenic. [b] Delisted for use in food in 1959. [c] Delisted in 1966 because of "insufficient economic importance". [d] Delisted in 1976.

Table 3. Comparison of Quantities of FD&C Dyes Certified for Use in the United States by the FDA in 2007 *(36)*

Dye	Mass, kg	Percentage of Total
Blue 1	240,654	4.1
Blue 2	208,646	3.6
Green 3	5739	0.1
Red 3	79,746	1.4
Red 40	2,330,576	40.0
Yellow 5	1,558,756	26.7
Yellow 6	1,406,060	24.1
Totals	5,830,177	100

The preceding information provides the necessary background for incorporating food dye experiments into the undergraduate laboratory curriculum. This information will enable students to make connections between chemical concepts and their application in the determination of synthetic dyes found in food colorings from around the world.

Paper Chromatography of Synthetic Food Dyes

Simple paper chromatography experiments were performed using 7.5 × 13.5-cm pieces of Whatman No. 1 chromatography paper and 7 mL of 0.10 wt % aqueous table salt as the solvent in 250-mL beakers covered with Petri dish or watch glass covers *(38)*. The food dyes were applied to the chromatography paper using wooden toothpicks bought at a grocery store. The seven FD&C dyes were obtained from Rainbow Colors, LLC.[4] The seven other food dyes studied were obtained from Sigma-Aldrich

Table 4. Representative Characteristics of Synthetic Food Dyes Identified in Food Colors from around the World

Dye	FDA Dye Name	Common Dye Names	Molar Mass	E Number[a]	CAS Number	Color Index Number
1	FD&C Blue 1[b]	Brilliant Blue FCF food blue 2	792.86	E-133	3844-45-9	42090
2	FD&C Blue 2[b]	Indigo Carmine food blue 1	466.36	E-132	860-22-0	73015
3	FD&C Green 3[b]	Fast Green FCF food green 3	808.86	—	2353-45-9	42053
4	FD&C Red 3[b]	Erythrosine food red 14	879.86	E-127	16423-68-0	45430
5	FD&C Red 40[b]	Allura Red AC food red 17	496.43	E-129	25956-17-6	16035
6	FD&C Yellow 5[b]	Tartrazine food yellow 4	534.37	E-102	1934-21-0	19140
7	FD&C Yellow 6[b]	Sunset Yellow FCF food yellow 3	452.37	E-110	2783-94-0	15985
8	FD&C Red 2[c]	Amaranth food red 9	604.47	E-123	915-67-3	16185
9	FD&C Red 4[c]	Ponceau SX food red 1	480.43	—	4548-53-2	14700
10		Ponceau 4Rc food red 7[c]	604.47	E-124	2611-82-7	16255
11		Carmoisine food red 3[c]	502.434	E-122	3567-69-9	14720
12	D&C Yellow 10[b]	Quinoline Yellow food yellow 13	579.42	E-104	8004-92-0	47005
13		Green S food green 4[c]	576.62	E-142	3087-16-9	44090
14		Patent Blue V (violet) food blue 5[c]	582.66	E-131	20262-76-4	42051

[a] Identification number for dyes approved for use in food by the European Economic Community. [b] Approved by the U.S. FDA and also used around the world. [c] Banned by the U.S. FDA, but used in other countries.

Table 5. Distribution of Synthetic Dyes Permitted for Use in Foods in Countries around the World

	Country, Total Number of Food Dyes Permitted by That Country											
Dye Name	United States, 7	Canada, 9	England, 15	France, 15	Ireland, 15	Spain, 15	Japan, 12	Australia, 11	New Zealand, 11	Germany, NA[a]	China, NA[a]	Singapore, NA[a]
Brilliant Blue FCF, B1	•	•	•	•	•	•	•[b]	•	•	•		•
Indigotine, B2	•	•	•	•	•	•	•	•	•			
Patent Blue V			•	•	•	•				•		
Fast Green FCF, G3	•	•					•					
Green S			•	•	•	•		•	•			
Erythrosine, R3	•	•	•	•	•	•	•[b]	•	•			•
Allura Red AC, R40	•	•	•	•	•	•	•	•	•			
Amaranth		•	•	•	•	•	•	•	•			
Carmoisine			•	•	•	•						•
Tartrazine, Y5	•	•	•	•	•	•	•[b]	•	•		•	•
Quinoline Yellow			•	•	•	•				•		
Sunset Yellow FCF, Y6	•	•	•	•	•	•	•	•	•		•	•
Ponceau 4R			•	•	•	•	•	•	•	•	•	•
Ponceau SX		•										
Phloxine B							•					
Rose Bengal							•					
Acid Red 53							•					
Red 2G			•	•	•	•						
Brilliant Black			•	•	•	•		•	•			
Chocolate Brown HT			•	•	•	•		•	•			

[a] Dyes identified in this research. Research did not yield a complete list of permitted dyes for Germany, China, and Singapore. [b] Lakes of these dyes also allowed.

and TCI America chemical companies (see the online supporting information for details). The pure dye standards were prepared as 0.50 wt % aqueous solutions, and were stored in 30-mL dropping bottles. The blue, red, yellow, and green food colors from Germany were pastes, and made up as 2.0 wt % aqueous solutions. The food colors from China and Japan were powders, and were also made up as 2.0 wt % aqueous solutions. Because the aqueous solutions prepared from these pastes and powders were too dilute, these food color solutions had to be spotted 3−5 times in order to obtain clearly visible spots on the chromatography paper. All of the other food colors were purchased from grocery stores in their respective countries by the authors or friends.

Table 6 gives the retardation factor (R_F) values, using a 0.10 wt % aqueous table salt solution as the solvent, for the seven U.S. food dyes and the other seven dyes used globally.

The separation of the seven U.S. food dyes by paper chromatography using 0.10 wt % aqueous table salt as the solvent works well (see Table 6), except for Blue 1 and Green 3, which have virtually identical R_F values. The colors of these two dyes on the chromatograph paper are also very difficult to differentiate. The seven foreign food dyes are quite well distinguished by simple paper chromatography using the same developing solution. Amaranth and Ponceau 4R have similar R_F values, but Amaranth is distinctly more purple in color than Ponceau SX. When one compares all 14 dyes combined, Red 40 and Ponceau SX are difficult to distinguish because their colors are virtually identical, and their R_F values are similar. Blue 1, Green S, and Patent Blue V are similar in color on the chromatograph paper, but do have significantly different R_F values, which allows for careful differentiation. Yellow 5 and Quinoline Yellow are quite close in color, yet vary considerably in R_F values. It is also interesting to note that while Amaranth and Ponceau 4R are very similar structural isomers (see the online supporting information) and differ only by the location of one sulfonic acid salt group, their R_F values are quite different.

Table 6. Comparison of R_F Values for Synthetic Food Dyes Used in the United States and throughout the World

Dye	R_F Values	Spot Color Characterization
FD&C Red 3, Erythrosine	0.08	bright pink
FD&C Red 40, Allura Red AC	0.34	red
FD&C Yellow 5, Tartrazine	0.58	yellow
FD&C Yellow 6, Sunset Yellow	0.42	orange
FD&C Blue 1, Brilliant Blue FCF	0.93	bright blue
FD&C Blue 2, Indigo Carmine	0.25	dull blue
FD&C Green 3, Fast Green FCF	0.92	blue, slight green tinge
Amaranth	0.28	red, slight purplish tinge
Carmoisine	0.14	pinkish red
Green S	0.86	blue
Patent Blue V (violet)	0.76	blue
Ponceau 4R	0.70	pinkish red
Ponceau SX	0.25	red
Quinoline Yellow	0.18	yellow

Visible Spectroscopy of Synthetic Food Dyes

The 14 dyes listed in Table 4 were oven-dried and stored in a desiccator prior to use. Solutions were prepared using an analytical balance (four decimal places), volumetric pipets, and flasks. Using the Beer−Lambert law, $A = abc$ (a = absorption coefficients from Marmion, see ref *41* and b = 1 cm), aqueous solutions were prepared that gave absorbances near 1.0, in order to accurately determine wavelength maxima and absorption coefficients. Visible spectra were recorded on a GBC UV/vis 918 instrument (GBC Scientific Equipment, Inc.) between 350 and 700 nm. Macro-cuvettes made of methacrylate were used for holding the solutions.

Table 7 gives the absorption maxima and absorption coefficients for the seven U.S. food dyes, and Table 8 reports the same characteristics for the seven foreign food dyes. The absorption maxima (λ_{max}) for U.S. dyes determined in this study are very close to those given by McKone *(16)* with the exception of Yellow 5. It is also interesting to note that the λ_{max} for Yellow

Table 8. Comparison of Absorption Maxima and Absorption Coefficient Values for Foreign Food Dyes

	Absorption Maxima, λ_{max} (nm)		
Dye	This Study	Literature Values	Absorption Coefficient (L g^{-1} cm^{-1})
Amaranth	521.6	520[a]	41.1
Carmoisine	516.0	515[b]	151
Green S	635.2	635[c]	113
Patent Blue V	637.6	NA[d]	178
Ponceau 4R	507.2	506[e]	36.0
Ponceau SX	501.6	500[f]	53.5
Quinoline Yellow	410.4	412[b]	91.6

[a] *Merck Index*, 13th ed. [b] *Aldrich Handbook of Fine Chemicals,*, 2007−2008. [c] TCI Organic Chemicals, 2006−2007 catalog. [d] NA: not available. [e] Spectrum Chemicals, 2002−2003 catalog. [f] *Merck Index*, 13th ed., in 0.2 N ammonium acetate.

Table 7. Comparison of Absorption Maxima and Absorption Coefficient Values for U.S. FDA-Approved Food Dyes

	Absorption Maxima, λ_{max} (nm)				Absorption Coefficient (L g^{-1} cm^{-1})	
Dye	This Study	McKone *(16)*	*Merck Index (39)*	Aldrich *(40)*	This Study	Marmion *(41)*
FD&C Red 3	526.6	527	524	525	114	110
FD&C Red 40	500.0	500	NA[a]	504	58.0	55.6
FD&C Yellow 5	426.4	422	NA[a]	NA[a]	57.7	53
FD&C Yellow 6	481.6	480	NA[a]	482	54.0	55
FD&C Blue 1	628.8	630	630	NA[a]	169	164
FD&C Blue 2	609.8	610	NA[a]	608	51.5	47.8
FD&C Green 3	624.0	625	628	NA[a]	165	156

[a] NA: not available.

5, which is the second-most commonly used dye in the United States (see Table 3), was not found in the *Merck Index (39)* or the *Aldrich Handbook of Fine Chemicals (40)*. The absorption coefficients determined in this study are also very similar to those of Marmion *(41)*. The visible spectra of the U.S. food dyes have been recently published *(20)*.

The absorption maxima for the foreign food dyes are also quite similar to those given in various chemical catalogs (see Table 8). The authors were unable to find absorption coefficients for the foreign dyes. From the standpoint of visible spectroscopy, the only dyes that would be difficult to separate based solely on their λ_{max} values would be Red 40 and Ponceau SX (Red 4), both having a λ_{max} of approximately 500 nm. It should be noted that Ponceau SX does have a secondary peak near 405 nm. Because the paper chromatography results for Red 40 and Ponceau SX are similar in both color and R_F values, one could positively identify these two dyes based on the secondary peak (405 nm) for Ponceau SX.

Conclusion

Artificial food colorants are commonly used around the world to enhance the appearance of food. Making connections for students using food dyes greatly enhances their understanding of paper chromatography and visible spectroscopy, as evidenced by our ongoing interactions with our students in organic, quantitative analysis, and biochemistry courses. When the U.S. dyes are used as the standards, and foreign dyes as unknowns, true inquiry experiments can be easily performed by students in introductory chemistry laboratories.

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Notes

1. Sudan I and Butter Yellow were withdrawn after being on the list for only six months after it was discovered that each caused dermatitis. Butter Yellow was later found to be carcinogenic.
2. A "lake" is a special type of color additive prepared by precipitating a dye onto an insoluble material, such as alumina.
3. Research did not yield a complete list of permitted dyes for Germany, China, and Singapore.
4. A food dye kit containing 2 g of each of the seven FD&C dyes was obtained from Rainbow Colors, LLC, 206 Eastwood Circle, Windsor, CT 06095, 860−219−9819; http://www.rainbowcolorsct.com/ (accessed Oct 2010).

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Supporting Information Available

Chemical structures of 14 food dyes; sources for obtaining non-FD&C dyes. This material is available via the Internet at http://pubs.acs.org.